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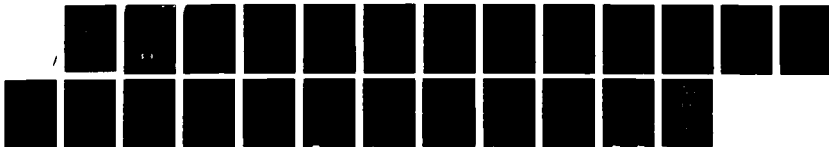
INCLUSION COMPLEXES OF DIISOPROPYL FLUOROPHOSPHATE WITH
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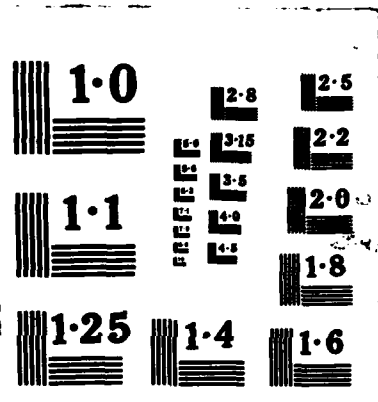
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INCLUSION COMPLEXES OF DIISOPROPYL FLUOROPHOSPHATE WITH CYCLODEXTRINS

K. S. Hall, R. A. Nissan, R. L. Quintana, and R. A. Hollins

Chemistry Division, Research Department

Naval Weapons Center, China Lake, CA 93555

Running Title: DFP Inclusion in Cyclodextrins

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ABSTRACT

Inclusion complexes of diisopropylfluorophosphate with α -, β -, and γ -cyclodextrins (CD) have been studied by ^1H and ^{31}P NMR methods. Binding constants (K) for the guest-host complex are determined for β - and γ -CD. Both NMR methods lead to the conclusion that β -CD forms the tightest complex with DFP, an order of magnitude tighter than γ -CD. We also conclude that α -CD does not form an inclusion complex with DFP.

INTRODUCTION

The hydrolysis of diisopropylfluorophosphate (DFP) and other phosphorus esters has been of great interest (1). The discovery of the enzyme DFPase shows great promise for the hydrolysis of DFP (2), but a major problem with enzymes is their poor stability and limited availability. The use of enzyme mimics for these type of hydrolyses would relieve the stability and availability problems. One of the best known molecules used in biomimetic studies is cyclodextrin (CD) (3). We have studied the inclusion complexes of DFP with α -, β -, and γ -CD using nuclear magnetic resonance (NMR) techniques. The first step towards catalytic hydrolysis of DFP will be to demonstrate binding in the CD cavity. Future work will deal with functionalized CD molecules.

Cyclodextrins are cyclic oligomers of glucose. The most common CDs are the hexamer (α), heptamer (β), and the octamer (γ) which have a molecular weight around 1000 (Fig. 1). Cyclodextrins have a conical doughnut shape with hydroxyl groups on the outer surface while the cavity is hydrophobic, similar to what would be expected for a miniature enzyme. The solubility of CD in water is good, making the inclusion of various apolar molecules possible (3). In solution a 1:1 molar ratio guest-host complex is usually formed. This interaction can enable enhanced reaction rates when CD or a functionalized CD is a reactant. Functional groups can be attached directly to the CD molecule making it catalytically active; first an inclusion complex must be formed with the guest molecule.

The first report on the interaction of DFP and CDs was a study of kinetics and thermodynamics of the reaction of DFP with α -CD in aqueous alkaline media (4). Calorimetric measurements were used to indicate that an inclusion complex was formed. A recent article on the interaction of phosphorus containing aromatic compounds with α -CD indicated that the addition of the phosphate ester group makes the molecule too bulky to form an inclusion complex (5). This work addresses the question of whether DFP forms an inclusion complex with α -, β -, or γ -CD.

The characterization of the guest-host inclusion complex can give valuable information as to what type of CD derivative should be synthesized to enable greater success in obtaining the catalytic activity desired. The use of ^1H NMR to study the inclusion of aromatic hydrocarbons by CD has been studied previously (6). Peaks for the H-3 and H-5 atoms of α -CD, which are directed toward the interior of the CD cavity (Fig. 2), showed significant chemical shifts when substituted benzoic acids were added to CD solutions in D_2O . Atoms which reside on the exterior of the cavity, H-1, H-2, H-4, had only marginal shifts. The large upfield shift for the H-3 and H-5 atoms is attributed to the anisotropic shielding effect of the aromatic rings of the benzoic acids included in the CD cavity.

The change in chemical shift of the internal protons due to the inclusion of a guest molecule can help determine the guest-host molar ratio for the complex, the binding constant, K , and the molecular disposition of guest compounds. We have studied the DFP/CD complex by ^1H NMR and ^{31}P NMR. The ^{31}P NMR was helpful in confirming the ^1H NMR data and also in clarifying the molecular disposition of DFP in the CD cavity.

EXPERIMENTAL

The following materials were used: α -CD, β -CD, γ -CD (all from Sigma Chemical Co., St. Louis, Mo.) and DFP (Aldrich). All CDs were recrystallized twice from water.

For the ^1H NMR studies, all CDs were dried in a vacuum oven at 60°C for 24 hours. For the binding studies, all solutions were made with 99.9% D_2O to give a final CD concentration of 4.4 mM; varying amounts of DFP were added. Since the possibility of reference binding to the CD could not be excluded, no internal ^1H NMR reference was added. An external ((3-trimethylsilyl)-1-propane sulfonic acid (TSS) in D_2O) sample was used.

The spectra were recorded on a Nicolet NT-200 spectrometer operating at 200 MHz for ^1H acquisition and 81 MHz for ^{31}P acquisition. In aqueous solution, the resonances from only the nonexchanging hydrogens are detected. The assignments of the CD spectra were determined previously (6,7).

The chemical shift value of the i^{th} hydrogen of CD is referenced to the chemical shift of the proton on C_1 , H-1. The external H-1 proton was chosen as a reference since DFP inclusion would have a minimal effect on the H-1 chemical shift. Any changes in the $\Delta\delta_i$ for inner-surface hydrogen is because of the added guest and may be related to the nature of the adduct and not to pH or solvent effects. Figures 3 through 5 show the $\Delta\delta$ of α -, β -, and γ -CD, respectively, over the molar ratio of DFP/CD (R is the molar ratio of DFP to CD). Table 1 shows the change in $\Delta\delta$ over the complete range of molar ratios of DFP/CD (R). The range of R was only 2.0 for α -CD since only a 1:1

inclusion complex was considered feasible. The range was extended to 5.0 and 10.0 for β - and γ -CD respectively since a 2:1 complex was a possibility for these two CDs.

For the ^{31}P NMR experiments, a 5 mL 2.27 mM DFP (in D_2O) sample was used with the addition of varying amounts of solid CD. An external reference of 85% phosphoric acid was used. A graph of δP vs. R (where R is now the molar ratio of CD/DFP) for the three cyclodextrins is shown in Fig. 6 (δP is the average of the DFP doublet).

RESULTS AND DISCUSSION

^1H NMR Studies

The use of ^1H NMR for the detection of the inclusion of aromatic compounds by CDs has been demonstrated by other researchers (6, 8). These researchers have shown that if inclusion takes place, the screening environment produced by the ring current should be sensed by hydrogens on the inner surface (H-3 and H-5) of the CDs. With the same reasoning, we expected measurable screening of the internal protons even with the inclusion of a non-aromatic molecule such as DFP. Since there is no ring current effect, the amount of shielding the internal protons would experience by the inclusion of DFP was expected to be less than the inclusion of an aromatic. We also would expect that the outer protons would not be affected by the inclusion of DFP, as was found with the inclusion of aromatic substances.

α -Cyclodextrin binding studies with DFP are shown as a plot of the values of $\Delta\delta$ vs. R in Fig. 3. There was no change in $\Delta\delta$ by the addition of DFP, indicating that an inclusion complex is not formed. All $\Delta\delta$ are constant to within 0.010 ppm over the complete range of R. It may be that the α -CD cavity is too small for inclusion of the bulky isopropyl groups of DFP.

Figure 4 shows the β -CD ^1H NMR binding studies which proved to be more interesting than the α -CD data. Like the α -CD, the $\Delta\delta_2$ and $\Delta\delta_4$ remain almost constant within 0.010 ppm for the complete range of R. However, a large change in shielding is experienced by both the H-3 and H-5 atoms. The $\Delta\delta_3$ initially at R = 0 is 1.104 ppm and increases to 1.143 ppm. $\Delta\delta_5$ is initially at 1.232 ppm and also increases to an upper limit of 1.288 ppm. The H-6 hydrogens which are located on the smaller end (primary hydroxyl side) of CD and directed inwards in the gauche gauche conformation (6) are also affected; $\Delta\delta_6$ increased slightly with a total change of 0.016 ppm.

The ^1H NMR data of γ -CD binding to DFP is displayed in Fig. 5. $\Delta\delta_2$, $\Delta\delta_4$, and $\Delta\delta_6$ are all constant within 0.011 ppm. Both $\Delta\delta_3$ and $\Delta\delta_5$ increase with the addition of DFP up to 1:1 molar ratio. The total increase for the range of R = 10 for $\Delta\delta_3$ was 0.033 ppm and $\Delta\delta_5$ was 0.049 ppm which is similar to β -CD. There appears to be no break at 1:1 for the complex as seen with β -CD. This may indicate loose binding of DFP with γ -CD as compared to the tighter binding observed with β -CD.

We can assume that the $\Delta\delta_1$ for R = 0 are the values for the water-included adduct or the empty CD and those at large R represent the DFP-CD

complex (4). With this assumption and that a 1:1 complex is formed, the $\Delta\delta$ data can be used to estimate the binding constant, K , for the DFP-CD adduct. Table 2 shows the $\Delta\delta$ values of complexed and uncomplexed β -CD and γ -CD for H-3 and H-5 atoms and also the calculated binding constants associated with these atoms. For β -CD, K is $1.4 \times 10^3 \text{ M}^{-1}$ and $1.25 \times 10^3 \text{ M}^{-1}$ for H-5 and H-3, respectively. The K values for γ -CD are 69.5 M^{-1} and 76.0 M^{-1} for H-5 and H-3. The calculated values of K using H-5 or H-3 agree nicely for both β - and γ -CD. The H-5 protons in both CDs experience a greater interaction with DFP than H-3. This may be due to a tighter fit of the isopropyl groups of DFP into the smaller part of the CD cavity. The δ of H-6 does not move significantly in either β - or γ -CD, indicating that the DFP molecule does not enter from the smaller end of the cavity. The change in ^1H chemical shift data indicates that DFP may enter from the larger opening and fit more snugly into the smaller part of the cavity. From the ^1H NMR, the molecular disposition of DFP has not yet been determined, but it is expected that the isopropyl groups are in the cavity and the phosphorus is outside of the larger end of both β - and γ -CD.

^{31}P NMR Studies

The ^{31}P NMR studies examine the guest molecule instead of the host molecule, the reverse of the ^1H NMR experiments. These experiments were done by keeping the DFP concentration constant and by adding solid CD to the NMR sample. To interpolate the data a molar ratio of CD to DFP (R) had to be used. Figure 6 shows the change in chemical shift of the phosphorus in DFP (δP) over a range of R from 0.0 to 4.0 for all three CDs. This data indicates

a 1:1 complex for both β - and γ -CD. In both cases we see a break at approximately 1:1 and then a steep increase in chemical shift as more CD is added. Our data indicates that the phosphorus nuclei are not included in the cavity and are influenced by CD solution effects at molar ratios above 1.5:1. A theoretical chemical shift maximum associated with only complexation must be used to determine the binding constant.

The binding constants, K , for β - and γ -CD were calculated using the uncomplexed ^{31}P chemical shift of -9.885 ppm which is the value for DFP with α -CD below a 1:1 molar ratio. This value was chosen because it takes into account any change in chemical shift due to the addition of a sugar that does not form an inclusion complex with DFP. The complexed ^{31}P chemical shift that was used for the determination of K was extrapolated to be -10.00 ppm for β -CD and -9.975 ppm for γ -CD. Assuming a 1:1 complex, $K_{\beta\text{-CD}} = 1.84 \times 10^3 \text{ M}^{-1}$ and $K_{\gamma\text{-CD}} = 169 \text{ M}^{-1}$. These K values are not as accurate as those values calculated using ^1H NMR, but they do agree qualitatively.

CONCLUSION

Understanding the orientation and type of binding of CD-guest molecules can give insight into the engineering of functionalized CDs. Since it appears not to make an inclusion complex with DFP, these results indicate that α -CD is not a good candidate for derivatization. ^1H NMR techniques are better suited than calorimetric methods for studying inclusion complexes with CDs (4). Thermochemical studies can be more subject to solvent and pH effects than are the corresponding NMR studies.

Both β - and γ -CD are able to form a 1:1 inclusion complex with DFP. The molecular disposition of DFP in the cavity of both β - and γ -CD appears to be the same. The isopropyl groups are in the cavity and the phosphorus is outside of the secondary hydroxyl end of CD; this orientation is displayed in Figure 7.

From this data it appears that functional groups for the hydrolysis of DFP should be attached to the 2° hydroxyl end of β - and γ -CD. This would enable the reacting groups to be in close proximity to the P-F bond. At this moment it is difficult to predict which CD, β or γ , will have the best catalytic rate once derivatized. Even though β -CD has much tighter association with DFP than γ -CD, this does not automatically mean it would have higher activity. Previous work has shown that the catalytic activity of CDs can be independent of association constants (3). Hopefully, the work presented in this paper will help guide the design of enzyme mimics for the catalytic transformation of phosphorus esters.

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FIGURE CAPTIONS

1. Shape and structure of the CD cavity.
2. The approximate location of the nonexchangeable protons of CD.
3. ^1H NMR results of α -CD with DFP displayed as a graph of ppm vs. R, where R is the molar ratio of DFP to α -CD.
4. ^1H NMR results of β -CD with DFP displayed as a graph of ppm vs. R, where R is the molar ratio of DFP to β -CD.
5. ^1H NMR results of γ -CD with DFP displayed as a graph of ppm vs. R, where R is the molar ratio of DFP to γ -CD.
6. ^{31}P NMR results of α -, β -, and γ -CD with DFP displayed as a graph of ppm vs. R where R is the molar ratio of CD to DFP.

TABLE 1

The change in ^1H NMR $\Delta\delta_i$ (ppm) for α -, β -, and γ -CD over R.^a

R ^b	H-2	H-3	H-4	H-5	H-6
α -CD	0.006	0.010	0.009	0.003	0.010
β -CD	0.008	0.039	0.009	0.056	0.016
γ -CD	0.011	0.033	0.011	0.049	0.004

^a The R is the range of DFP/CD molar ratio that the change in $\Delta\delta_i$ was measured. $\Delta\delta_i$ is the chemical shift of the i^{th} proton in reference to H-1 proton.

^b R for α -CD from 0.0 to 2.0; R for β -CD from 0.0 to 4.0; R for γ -CD from 0.0 to 10.0.

TABLE 2

Complexed and uncomplexed Δ chemical shifts ($\Delta\delta$) for H-3 and H-5 for β -CD and γ -CD. Binding constants for β -CD and γ -CD.

	β -CD		γ -CD	
	H-3	H-5	H-3	H-5
$\Delta\delta_u^a$	1.104	1.232	1.174	1.271
$\Delta\delta_c^b$	1.143	1.288	1.208	1.320
$K (M^{-1})^c$	1.250	1.400	69.5	76.0

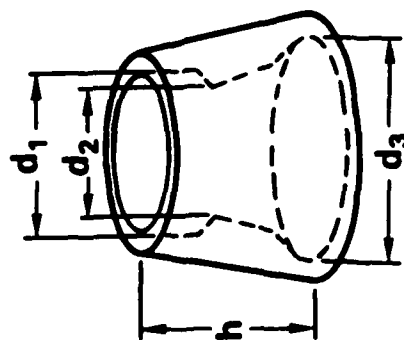
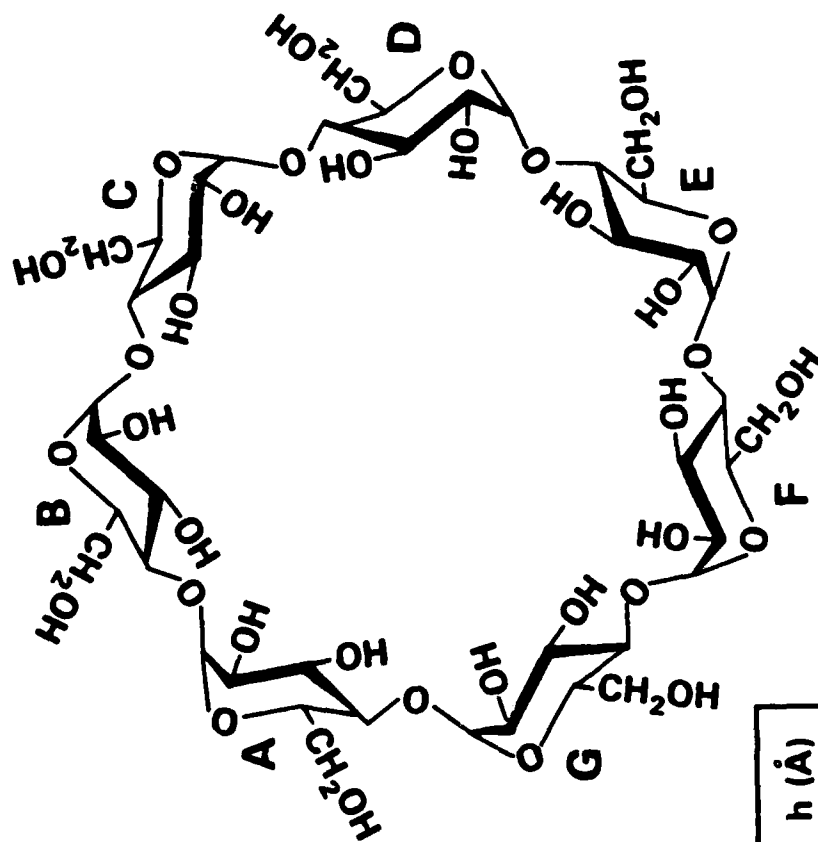
^a Uncomplexed CD.

^b Complexed DFP/CD (values taken from Fig. 2 and 3).

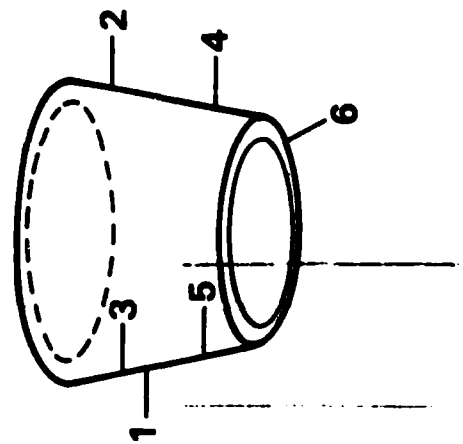
^c K was determined by the end chemical shift

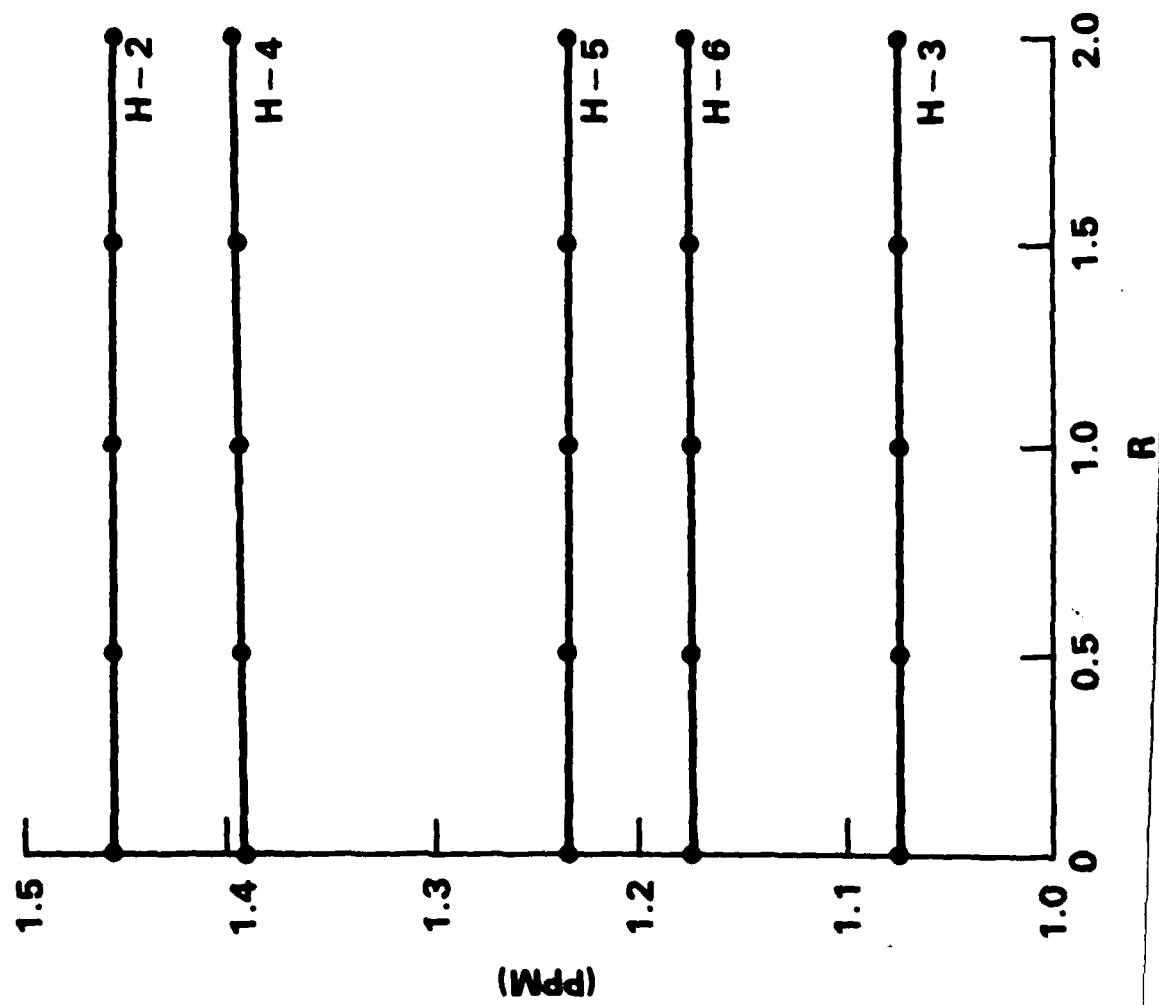
equation: $\Delta\delta_r = \Delta\delta_u N_u + \Delta\delta_c N_c$. $\Delta\delta_r = \Delta\delta$

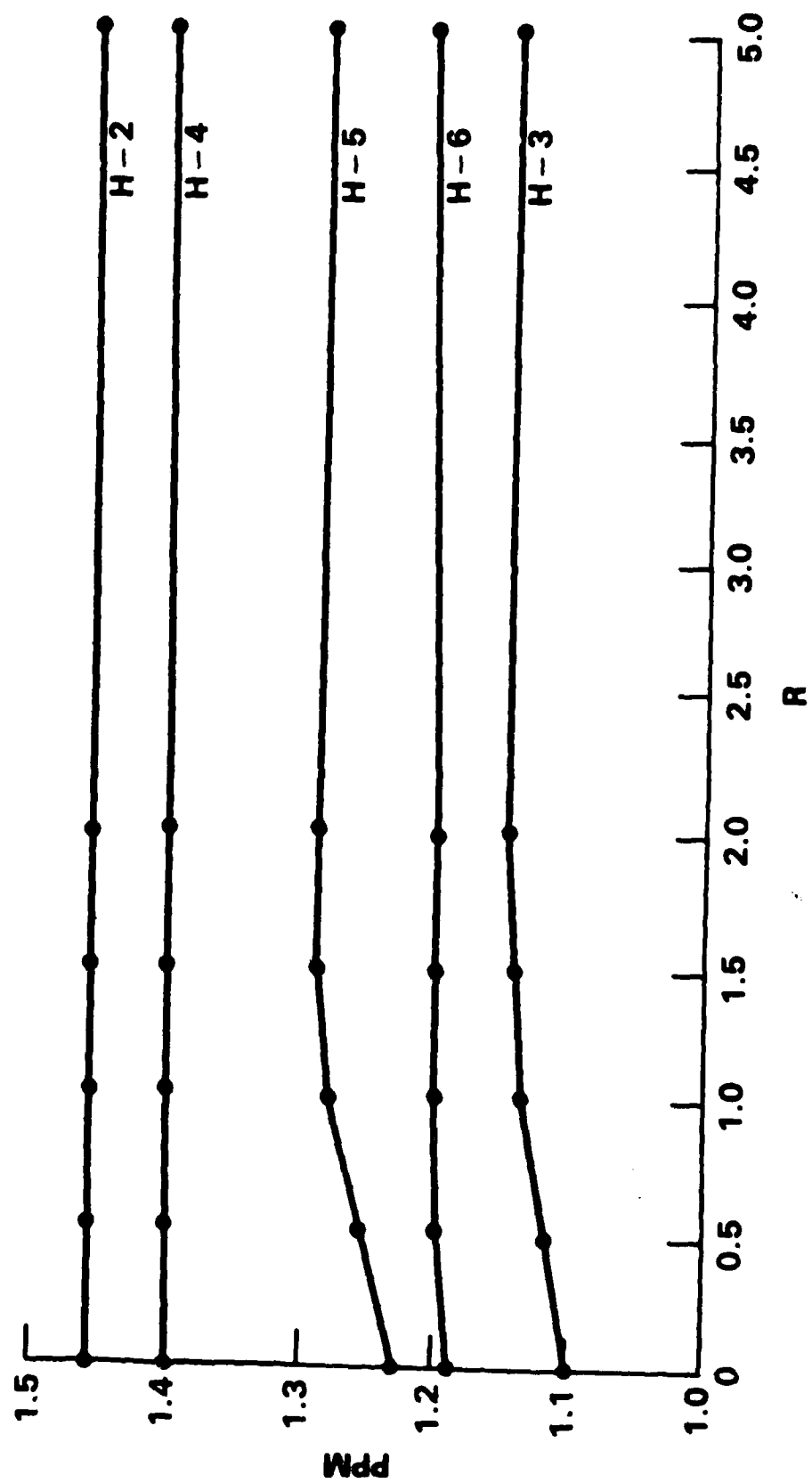
at a specific R (DFP/CD molar ratio); N_c = mole fraction of complexed CD; N_u = mole fraction of uncomplexed CD.

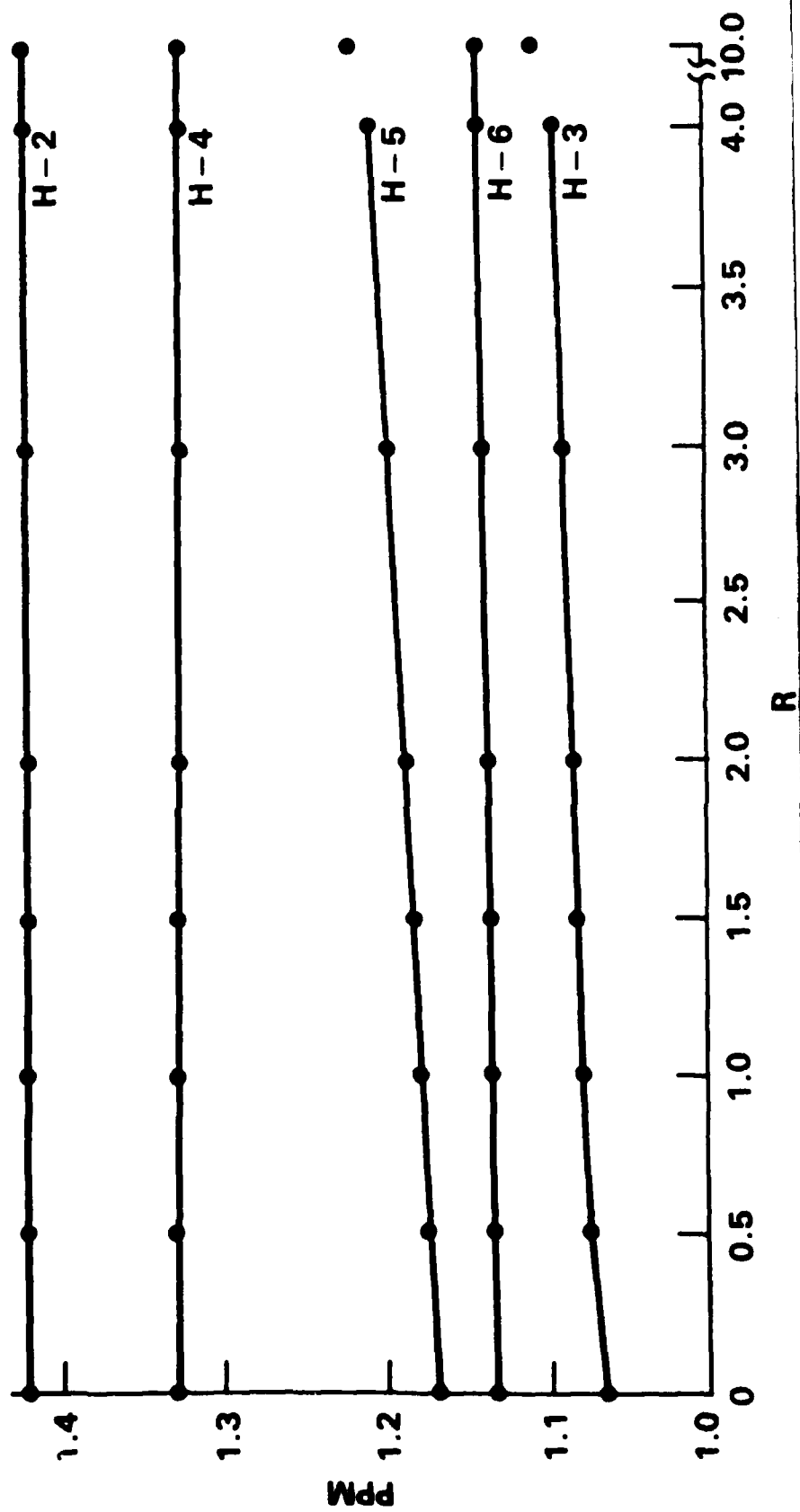


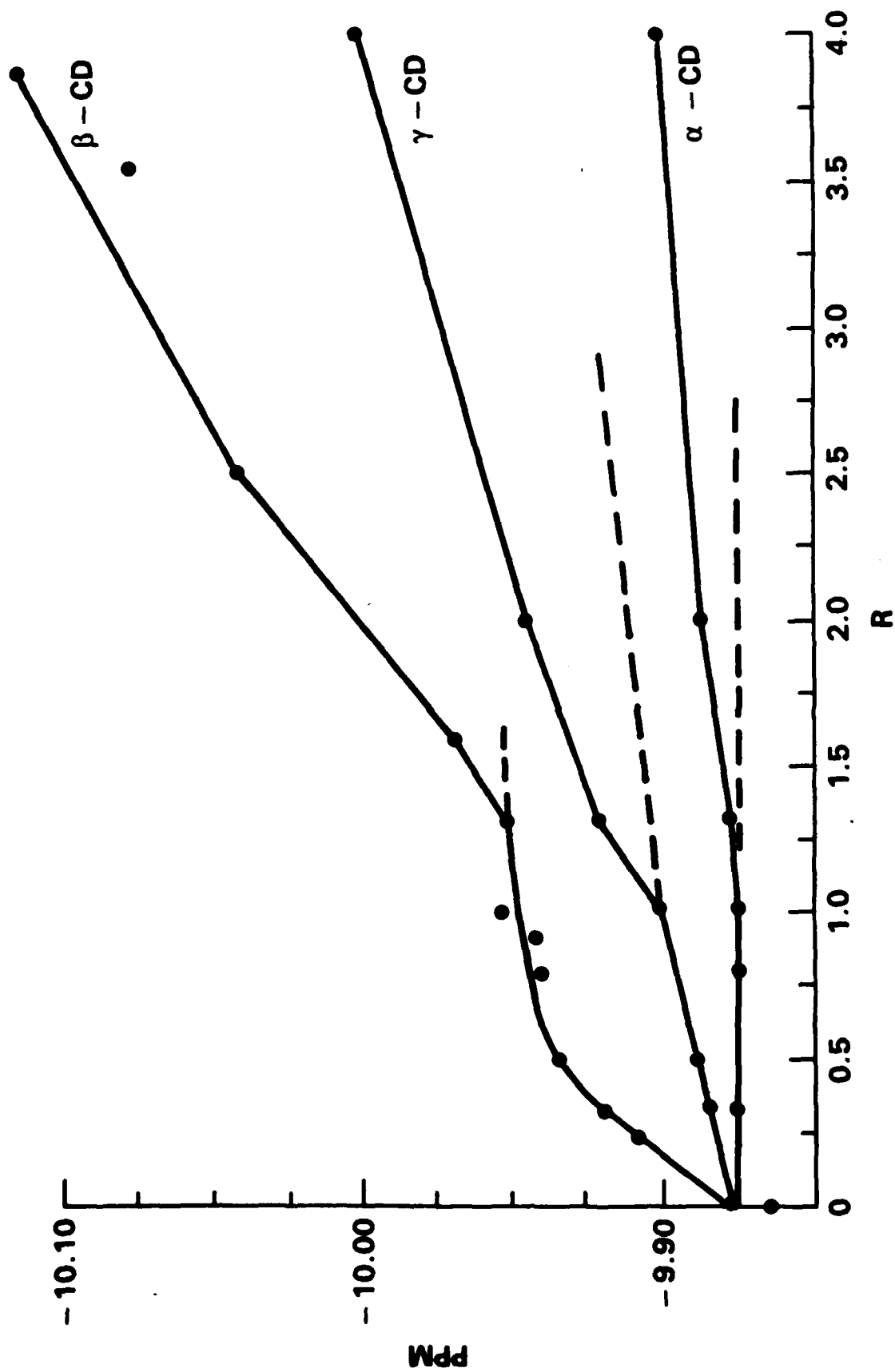
CYCLO - DEXTRIN	d_1 (Å)	d_2 (Å)	d_3 (Å)	h (Å)
α	5.6	4.2	8.8	7.8
β	6.8	5.6	10.8	7.8
γ	8.0	6.8	12.0	7.8

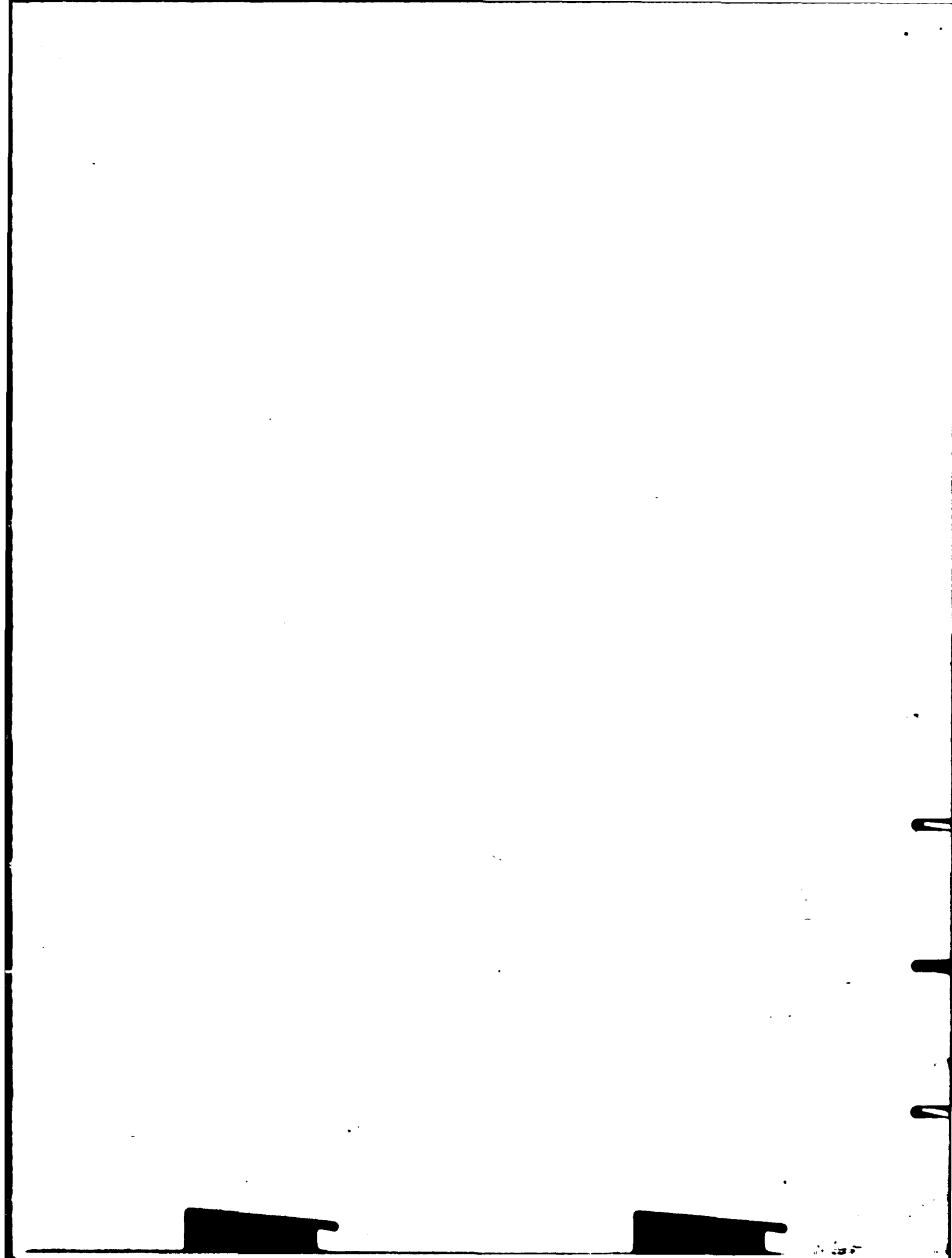












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